(12) UK Patent Application (19) GB (11) 2 414 021 (13) A

(43) Date of A Publication 16.11.2005

(21) Application No: 0410383.4

(22) Date of Filing: 10.05.2004

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(51) INT CL[7]:
C08J 9/40 , A61L 2/08 15/32

(52) UK CL (Edition X):
C3V VET
C3W W123

(56) Documents Cited:
EP 1153622 A1 **EP 0568334 A1**
US 5399361 A **US 20020187194 A**
US 20020164322 A
WPI Abstract Accession No. 2003-742415 & RU 2198684
SURGIFOAM (RTM)

(58) Field of Search:
UK CL (Edition W) **C3V**
INT CL[7] **A61L, C08J**
Other: **ONLINE: WPI, JAPIO, EPODOC**

(54) Abstract Title: **Absorbable haemostatic materials**

(57) A method of making an absorbable gelatin sponge, comprising the steps of: (a) dispersing gelatin in a solvent to form a gelatin dispersion; (b) drying the gelatin dispersion to produce a gelatin sponge; (c) crosslinking the gelatin to render it substantially insoluble in water; followed by (d) irradiating the sponge with ionizing radiation, such as gamma radiation, to reduce its resorption time in vivo. Gelatin sponges obtainable by the method of the invention are also provided. The sponge may be further treated with a C_2-C_{10} alcohol carrying a therapeutic agent to impregnate the sponge with that agent.

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ABSORBABLE HAEMOSTATIC MATERIALS

The present invention relates to absorbable haemostatic materials and to methods for the manufacture thereof.

Gelatin sponges have been used for many years as absorbable surgical hemostats. Gelatin sponges are available from Johnson & Johnson under the Registered Trade Marks SURGIFOAM or SPONGISTAN. The sponges are produced by the steps of: dissolving gelatin in water, foaming the solution by blowing filtered gas through the solution, spreading or casting the foam into the desired shape, drying and dehydrothermally cross-linking the foam at about 134°C, followed by sterilizing the resulting sponge with electron beam or heat. The dehydrothermal cross linking step results in a sponge having a resorption time *in vivo* of 2 to 4 weeks, which is undesirable for many surgical hemostasis applications. The long resorption time is retained following sterilization with heat.

US-A-2558395 describes gelatin sponges containing thrombin as an additional hemostatic agent. The thrombin is added to an aqueous solution of gelatin, which is then freeze-dried to form a soluble gelatin sponge containing thrombin. WO95/27517 describes a similar process for incorporating Gentamycin into gelatin sponges. The resulting sponges may be sterilized by irradiation. JP-A-55084167 describes gelatin sponges having medicated oily droplets incorporated therein. None of these gelatin sponges is crosslinked, and consequently they all dissolve promptly *in vivo*, which is undesirable for many surgical hemostasis applications.

In a first aspect, the present invention provides a method of making an absorbable gelatin sponge, comprising the steps of : (a) dispersing gelatin in a solvent to form a gelatin dispersion; (b) drying the gelatin dispersion to produce a gelatin sponge; (c) crosslinking the gelatin to render it substantially insoluble in water; followed by (d) irradiating the sponge with gamma radiation.

The present inventors have found that irradiation of the sponge can be used to prepare a gelatin sponge that breaks down more rapidly in the presence of collagenase, without loss of hemostatic properties.

The crosslinked gelatin sponge is preferably made by the following process. Gelatin is dispersed in an aqueous solvent, preferably water, at a concentration typically about 1 to about 10wt.%, suitably about 2 to about 8wt.%, for example about 5wt%. The solution is optionally filtered. A gas such as filtered air is blown through the solution to produce a thick foam. The foam is then shaped, for example by spreading onto plates, before drying. The drying may be carried out by freeze drying, and/or by evaporation at elevated temperatures. These methods of forming the sponges are described in more detail in US-A-2558395 and WO-A-9527517, the entire contents of which are incorporated herein by reference.

An alternative second method for forming the gelatin sponges comprises dispersing or dissolving the gelatin in the aqueous vehicle, usually a suitably buffered water, at a solids concentration of from about 0.1wt% to about 5wt%, preferably about 0.3wt% to about 3wt%, followed by freezing the dispersion and carrying out freeze-drying or solvent drying on the frozen dispersion, for example as described in EP-A-1153622 or in EP-A-0838491, the entire contents of which are incorporated herein by reference.

The step of preparing the gelatin sponge further comprises the step of crosslinking the gelatin. The step of crosslinking may be carried out before, during and/or after the step of drying the dispersion. The crosslinking may be carried out by the addition of a chemical crosslinking agent such as dicyclohexyl carbodiimide or glutaraldehyde. The chemical crosslinking agent can be added to the dispersion of the gelatin, or it can be applied to the gelatin after drying. However, the use of such chemical crosslinking agents can increase the antigenicity of the sponge. Therefore, the process according to the invention preferably comprises a step of dehydrothermal crosslinking. That is to say, crosslinking that takes place spontaneously when the gelatin is heated during the drying of the dispersion. Preferably, the gelatin is heated to at least 30°C to achieve the desired dehydrothermal crosslinking, more preferably at least 50°C, and still more preferably at least 100°C. In certain embodiments the dehydrothermal crosslinking may take place at about 120° to about 140°C, for example about 134°C.

The moisture content of the gelatin sponge before, during and after irradiation is preferably less than about 15% by weight, usually less than about 10% by weight, for example about 2% to about 5% by weight. The sponge may be cast in any convenient shape, including tubes and sheets for use in surgery. In other embodiments of the method according to the invention, the sponge may be comminuted into smaller pieces, or into a powder, suitable for spreading over a wound to arrest bleeding. The step of comminuting may for example take place either before or after the step of irradiation. Suitably, the sponge may be in the form of a powder having 90% by volume in the size range of from about 50m to about 1000μm, as determined by laser diffraction.

The gelatin foams or sponges may be medicated by the addition of one or more therapeutic agents before or after the drying step. In the case of post-drying treatment, the present inventors have found that treating the sponges with therapeutic agents dispersed in water or methanol causes the sponges to collapse. However, use of a C_2-C_{10} alcohol enables therapeutic agents to be incorporated into the sponges after the dehydrothermal cross-linking step, without any substantial change in the physical appearance or properties of the sponge. The alcohol may suitably be a monohydric alcohol, for example a C_2-C_6 monohydric alcohol. Preferred alcohols include ethanol, propanol or isopropanol. Preferably, the alcohol consists essentially of ethanol. It has also been found that treating the sponges with the specified alcohols, without adding any therapeutic agent, can render the sponges antimicrobial.

The therapeutic agent is suitably selected from the group consisting of a reactive oxygen scavenger, an antimicrobial agent, an antioxidant dyestuff, a pain relieving agent, a growth factor or mixtures thereof.

The reactive oxygen scavenger may be selected from the group consisting of antioxidant phenol derivatives, vitamin E, methyl peroxide antioxidants, stilbenes, gallocatechins, ubiquinol, retinoids, vitamin A, vitamin C, N-acetyl cysteine, selenium and its compounds, zinc and its compounds, glutathione, carotenoids, papain, thioproline, albumin, chlorophyllin, and mixtures thereof.

The term "dyestuff" refers to a material that is useful as a colorant for textile materials, that is to say an organic compound that is strongly light-absorbing in the visible region 400-700nm. In certain embodiments, the antioxidant dyestuff is selected from the group consisting of aniline dyes, acridine dyes, thionine dyes, bis-naphthalene dyes, thiazine dyes, azo dyes, anthraquinone dyes, and mixtures thereof. For example, the antioxidant dyestuff may be selected from the group consisting of gentian violet, aniline blue, methylene blue, crystal violet, acriflavine, 9-aminoacridine, acridine yellow, acridine orange, proflavin, quinacrine, brilliant green, trypan blue, trypan red, malachite green, azacrine, methyl violet, methyl orange, methyl yellow, ethyl violet, acid orange, acid yellow, acid blue, acid red, thioflavin, alphazurine, indigo blue, methylene green, and mixtures thereof.

The antioxidant dyestuff may suitably be present in the gelatin sponge according to the invention in an amount of from about 0.05% to about 5wt.%, typically about 0.2 to about 2wt.% based on the weight of the product material.

The antimicrobial agent may be selected from the group consisting of antiseptics and antibiotics and mixtures thereof. Suitable antibiotics include peptide antimicrobials (e.g. defensins, Magainin, synthetic derivatives of them); antibiotics such as gentamycin, tetracycline, penicillins, terramycins, erythromycin, bacitracin, neomycin, polymycin B, mupirocin, clindamycin and mixtures thereof. Suitable antiseptics include silver sulfadiazine, chlorhexidine, povidone iodine, triclosan, other silver salts and colloidal silver, sucralfate, quaternary ammonium salts and mixtures thereof.

The preferred therapeutic agent is Triclosan. Triclosan (2,4,4'-trichloro-2'-hydroxy diphenyl ether) is a well-known highly effective broad spectrum antimicrobial agent for topical applications, with a wide range of efficacy. Triclosan is thought to act specifically on the fabI gene product fatty acyl reductase (Type II) of the bacterial fatty acid biosynthesis system. This is distinct from the mammalian system, and consequently the Triclosan has low toxicity to mammalian cells. Triclosan is commercially available under the trade name Irgasan (Ciba-Geigy Limited) e.g. Irgasan DP300. It has been found by the present inventors that the antimicrobial activity of triclosan incorporated into gelatin sponges is substantially unaffected by gamma irradiation.

Suitably, the antimicrobial agent may be incorporated into the sponges at concentrations of between about 0.1-30% by weight, more suitable from about 0.5-15% by weight, and preferably between about 1-5% by weight based on the weight of the product material.

The pain relieving agent may be selected from the group consisting of an anaesthetic, an analgesic, an antiinflammatory or mixtures thereof. Suitable anaesthetics include lidocaine or novocaine. Suitable analgesics include non-steroidal anti-inflammatory drugs (NSAIDs). Suitable antiinflammatory agents include steroids such as prostaglandins.

Suitably, the pain relieving agent may be incorporated into the sponges at concentrations of between about 0.1-30% by weight, more suitably from about 0.5-15% by weight, and preferably between about 1-5% by weight based on the weight of the product material.

The growth factor may be selected from the group consisting of platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF-β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF), and mixtures thereof.

Suitably, the growth factor may be incorporated into the sponges at concentrations of between about 1ppm to about 1% by weight, more suitably from about 10ppm to about 1000ppm by weight based on the weight of the product material.

The therapeutic agent is typically dissolved or suspended in the C_2-C_{10} alcohol solvent at a weight concentration from about 0.1 to about 1x the final weight concentration desired in the sponge, since the sponges typically have a liquid absorbency of from about 1 to about 10g/g.

The methods of preparing medicated gelatin sponges typically comprise soaking the sponge in the C_2-C_{10} alcohol solution of the therapeutic agent so that the whole volume of the sponge is wetted by the solution, usually followed by draining the sponge and drying the sponge under mild conditions or under reduced pressure, suitably at temperatures below about 120°C, for example about 60°C to about 100°C. The sponge may be squeezed

to express surplus liquid before the step of drying. These methods can result in a sponge having the therapeutic agent substantially uniformly distributed through the sponge.

In other embodiments, the sponge may be sprayed or otherwise surface coated with the C_2-C_{10} alcohol solution of the therapeutic agent so that only a surface region of the sponge contains the therapeutic agent.

The step of irradiation with ionizing radiation is typically carried out with gamma radiation, for example Co^{60} gamma radiation. A suitable gamma ray dosage is from about 1 to about 50 kGy of Co^{60} radiation, for example about 5 to about 30 kGy. That is to say, suitably with about 0.1 to about 3 mRad of gamma radiation. Other forms of radiation such as microwaves may be considered.

Preferably, the methods according to the present invention further comprise a step of sterilizing the gelatin sponge before the step of gamma-irradiating the sponge. The pre-sterilizing may be carried out, for example, by heat in the conventional fashion. The gelatin sponge may also be packaged in a microorganism-impermeable package after, or preferably before, the step of gamma irradiation. In this way a sterile sponge may be produced even if the gamma irradiation dose is selected to be less than the 18-30kGy typically needed for complete sterilization by gamma irradiation only. The gamma ray dose can then be selected to give precisely the desired resorption time of the product.

In a second aspect, the present invention provides a crosslinked gelatin sponge, wherein the sponge is obtainable by a process according to the first aspect of the invention. All optional features described in relation to the first aspect may likewise be present in the sponge products of the second aspect. In particular, the crosslinked gelatin sponge preferably contains an antimicrobial agent, for example triclosan. The crosslinked gelatin sponge may be comminuted into small particles or powder. It may be sterile, and it may be packaged in a microorganism-impermeable container.

Specific embodiments of the present invention will now be described further, by way of example.

Example 1

A dehydrothermally crosslinked absorbable gelatin sponge that has previously been sterilized by heat (SURGIFOAM supplied by Johnson & Johnson) was cut into samples. The samples were treated as follows:

(a) no treatment (reference example)

(b) immersed in ethanol for 60 seconds, then removed and dried 6 hours at 100°C (reference example)

(c) Irradiated with 28 kGy of Co^{60} gamma irradiation; and

(d) Treatment with ethanol as in (b), followed by irradiation as in (c).

The resulting samples all showed substantially identical physical appearance. This shows the suitability of ethanol and higher alcohols as solvents for introducing active agents into dehydrothermally crosslinked gelatin sponges.

The swelling properties of the gelatin sponges were evaluated by the method of Lee et al. Eur. J. Pharm & Biopharm. 56, 407-412 (3003), the entire contents of which are incorporated herein by reference. Briefly, 30 x 30 mm SURGIFOAM dressings were cut and weighed. The dressings were then soaked in distilled water at 25°C for 60 minutes, 24 hours and 48 hours. After these time points, the dressings were placed on Whatman 1 filter paper, 30 seconds on one side and 30 seconds on the other. The dressings were then re-weighed and the swelling ratio were calculated according to the following equations:

$$\text{Swelling Ratio (\%)} = \frac{m_f - m_i}{m_i}$$

Where m_f is the mass of a dressings after soaking it in water and removing excess water, and m_i is the initial mass of the dressing before soaking. The measured swelling ratios were as follows:

Sample	Time = 1hr	Time = 24hr	Time = 48hr
(a)	14.1 ±0.1	12.6 ±1.5	10.4 ±2.9
(b)	13.3 ±0.5	11 ±1.2	9.4 ±3.7
(c)	13.6 ±0.05	11.9 ±0.3	8.8 ±3

(d)	14.4 ±0.07	10.9 ±2.6	8.3 ±1.4

It can be seen that the treatment with ethanol has little effect on absorbency properties. The gamma irradiation does not reduce the initial absorbency of the material, but the absorbency drops more sharply over time for the irradiated samples, which is consistent with more rapid breakdown of the irradiated materials *in vivo*.

The digestion of the gelatin sponges by a simulated wound fluid was studied as follows. A simulated wound fluid was prepared containing bacterial collagenase, at an activity of 1 relative fluorescent units per minute (RFU/min). 1cm x 1cm pieces of the sponges were placed into 5 ml of the simulated wound fluid at ambient temperature. The time taken for complete dissolution of the sponges was measured. The measured times for the samples (a) to (d) as defined above were: (a) >3hr, (b) 3hr, (c) <2hr, and (d) <2hr. This confirms that the gamma-irradiated samples should degrade faster *in vivo*.

A further experiment showed that the gamma-sterilized sponges (after ethanol treatment) disintegrated in distilled water after three days. The control sponges remained intact. This suggests the gamma irradiation treatment can increase the rate of dissolution in water alone.

Example 2

A further experiment on a sponge that had been treated with an ethanol solution containing glucose showed that the addition of glucose had negligible effects on the biodegradation times of the dressings.

Example 3

Samples of a dehydrothermally crosslinked absorbable gelatin sponge (SURGIFOAM supplied by Johnson & Johnson) were immersed in ethanol having Triclosan dissolved therein at concentrations of 10%, 1%, 0.1%, 0.01% and 0.001%w/v. After immersion for 60 seconds at ambient temperature, the sponges were weighed to determine the uptake of

the solution, and then dried at 60°C for 6 hours. The sponges were then sterilized with gamma radiation as for Example 1.

Zone of inhibition studies were carried out to assess the antimicrobial properties of the sponges against *staphylococcus* and *klebsiella*. The bacteria were seeded onto petri dishes in amounts of 2.9×10^7 (high density) and 2.9×10^6 (low density). The data showed that the gamma irradiation does not impair the antimicrobial properties of the triclosan in the gelatin sponges.

The sponges containing higher levels of triclosan exhibited greater physical integrity over time in the agar medium used for the zone of inhibition studies. This is a further potential advantage of triclosan loading.

The above examples have been described for the purpose of illustration only. Many other examples falling within the scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. A method of making an absorbable gelatin sponge, comprising the steps of:

(a) dispersing gelatin in a solvent to form a gelatin dispersion;

(b) drying the gelatin dispersion to produce a gelatin sponge;

(c) crosslinking the gelatin to render it substantially insoluble in water; followed by

(d) irradiating the sponge with ionizing radiation.

2. A method according to claim 1, wherein the step of dispersing comprises blowing gas into the solvent to form a gelatin foam.

3. A method according to claim 1 or 2, wherein the step of crosslinking comprises dehydrothermal crosslinking.

4. A method according to any preceding claim, further comprising the step of sterilizing the gelatin sponge before the step of irradiating.

5. A method according to any preceding claim, further comprising the step of comminuting the gelatin sponge.

6. A method according to any preceding claim, further comprising the steps of: treating the gelatin sponge with a dispersion of a therapeutic agent in a C_2-C_{10} alcohol; and drying the gelatin sponge to provide a medicated gelatin sponge.

7. A method according to claim 6, wherein the C_2-C_{10} alcohol comprises a C_2-C_6 monohydric alcohol, preferably ethanol, propanol or isopropanol.

8. A method according to claim 6 or 7, wherein the therapeutic agent is selected from the group consisting of a reactive oxygen scavenger, an antimicrobial agent, a pain relieving agent, a growth factor or mixtures thereof.

9. A method according to claim 8, wherein the therapeutic agent is selected from the group consisting of: peptide antimicrobials such as defensins and Magainin; antibiotics such as tetracycline, penicillins, terramycins, erythromycin, bacitracin, neomycin, polymycin B, mupirocin, clindamycin, and gentamycin; and antiseptics such as silver sulfadiazine, chlorhexidine, povidone iodine, triclosan, other silver salts and colloidal silver, sucralfate, quaternary ammonium salts; and mixtures thereof.

10. A method according to any preceding claim, wherein the ionizing radiation is gamma radiation.

11. An absorbable sponge comprising dehydrothermally crosslinked gelatin, wherein the sponge is obtainable by a process according to any preceding claim.

Application No: GB0410383.4 **Examiner:** Mr Jason Scott

Claims searched: 1-11 **Date of search:** 11 October 2004

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
Y	1-4, 10 and 11	WPI Abstract Accession No. 2003-742415 & RU 2198684 ROSSIJSKIJ NII GEMA; TOLOGII I TRANSFUZIOLOGII, 20.02.03, see abstract demonstrating the use of gamma-irradiation for treating a gelatin based foam.
X,Y	X: 1-5, 10 and 11; Y: 6-9	EP 1153622 A1 JOHNSON & JOHNSON MEDICAL LIMITED, see whole document and in particular column 5, lines 3-27 showing dispersion, drying, dehydrothermal cross-linking and irradiation.
Y	1-4, 10 and 11	SURGIFOAM (RTM) Gelatin Sponge available from Johnson & Johnson.
Y	6-9	US 2002/164322 A SCHAUFLER See whole document and in particular paragraphs 0090-0099 & 0351.
Y	6-9	US 2002/187194 A STIMMEDER See whole document and in particular paragraphs 0023-0031 & 0252.
Y	6-9	US 5399361 A AMGEN See whole document and in particular example 1.
Y	6-9	EP 0568334 A1 AMGEN See whole document and in particular example 1.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[W] :

C3V

Worldwide search of patent documents classified in the following areas of the IPC[07]

A61L; C08J

The following online and other databases have been used in the preparation of this search report

WPI, JAPIO, EPODOC